
1. SCIENTIFIC ABSTRACT OF THE PROTOCOL

This protocol is an extension of an earlier one in which a defective adenovirus vector (Ad2/CFTR-1) was applied to the nasal epithelium of three CF patients. In all three cases changes in electrolyte transport were detected following treatment with vector. No adverse effects that could be attributed to the adenovirus vector were detected. This protocol seeks to address the next key questions regarding the use of adenovirus vectors, first is it safe to administer virus multiple times and is it possible to obtain evidence of clinical as well as biochemical evidence of efficacy?

This protocol will use a second generation adenovirus vector named Ad2-ORF6/PGK-CFTR. This virus lacks E1 and in its place contains a modified transcription unit with the phosphoglycerate kinase (PGK) promoter and a poly A addition site flanking the CFTR cDNA. The PGK promoter is of only moderate strength but is long lasting and not subject to shut off. The E4 region of the vector has also been modified in that the whole coding sequence has been removed and replaced by ORF6, the only E4 gene essential for growth of Ad2 in tissue culture. This has the effect of generating a genome of 101% the size of wild type Ad2 and renders the vector more easy to grow in culture than Ad2-ORF6/PGK-CFTR.

The protocol involves production of Ad2-ORF6/PGK-CFTR-1 virus in 293 cells that have been extensively tested for adventitious agents, using a viral seed stock that has been similarly tested. Following purification and further testing, the Ad2/CFTR-1 stock will be applied directly to the nasal epithelium or to the maxillary sinus.

The protocol has two parts. In the first, Ad2-ORF6/PGK-CFTR will be applied in increasing doses (2×10^7 , 2×10^8 , 2×10^9 , and 2×10^{10} (twice)) to one nostril at approximately monthly intervals. Saline will be applied to the other nostril. In the second part of the protocol, virus will be applied to one submaxillary sinus up to seven times (5×10^8 (1x), 2.5×10^9 (up to 6x)). The contralateral sinus will act as a control. Five to ten patients will be recruited to each protocol, and patients will be carefully assessed for evidence of inflammation and virus replication following administration. Measurements of transepithelial voltage will be taken on the patients undergoing the nasal protocol and antibody titre to adenovirus will be studied. At the end of the first part of the protocol, biopsies of both nostrils will be taken. In the second part of the protocol, the sinus will be lavaged and treated with antibiotics and following the administrations of virus, evidence of reduced edema, bacterial infection and/or mucus production will be taken as indicators of clinical efficacy. Sinus biopsies will be taken at the end of the second part of the protocol.

Participants will be patients with CF who are at least 18 years old and have mild to moderate disease. We prefer patients homozygous for the common $\Delta F508$ mutation. We will require patients to be seropositive for adenovirus antibody and to have no evidence of respiratory viral infections for the prior two weeks. Following treatment, patients will remain isolated in the hospital for 24 hours.

The successful outcome of this protocol will add further to our knowledge of the safety and efficacy of adenovirus vectors and will be invaluable in the design of subsequent protocols targeted to the respiratory airways and of future generations of adenovirus vector.